Molecules and Cells

Minireview

Impaired Autophagic Flux in Glucose-Deprived Cells: An Outcome of Lysosomal Acidification Failure Exacerbated by Mitophagy Dysfunction

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Autophagy dysfunction is associated with human diseases and conditions including neurodegenerative diseases, metabolic issues, and chronic infections. Additionally, the decline in autophagic activity contributes to tissue and organ dysfunction and aging-related diseases. Several factors, such as down-regulation of autophagy components and activators, oxidative damage, microinflammation, and impaired autophagy flux, are linked to autophagy decline. An autophagy flux impairment (AFI) has been implicated in neurological disorders and in certain other pathological conditions. Here, to enhance our understanding of AFI, we conducted a comprehensive literature review of findings derived from two well-studied cellular stress models: glucose deprivation and replicative senescence. Glucose deprivation is a condition in which cells heavily rely on oxidative phosphorylation for ATP generation. Autophagy is activated, but its flux is hindered at the autolysis step, primarily due to an impairment of lysosomal acidity. Cells undergoing replicative senescence also experience AFI, which is also known to be caused by lysosomal acidity failure. Both glucose deprivation and replicative senescence elevate levels of reactive oxygen species (ROS), affecting lysosomal acidification. Mitochondrial alterations play a crucial role in elevating ROS generation and reducing lysosomal acidity, highlighting their association with autophagy dysfunction and disease conditions. This paper delves into the underlying molecular and cellular pathways of AFI in glucose-deprived cells, providing insights into potential strategies for managing AFI that is driven by lysosomal acidity failure. Furthermore, the investigation on the roles of mitochondrial dysfunction sheds light on the potential effectiveness of modulating mitochondrial function to overcome AFI, offering new possibilities for therapeutic interventions.

Keywords: autophagy, impairment of autophagy flux, lysosomal acidity, mitochondria, reactive oxygen species, V-ATPase

INTRODUCTION

The importance of functional autophagy in healthy tissues is suggested by the fact that various pathological conditions, such as neurodegenerative disorders including Alzheimer's and Huntington's diseases, are associated with autophagy dysfunction. In addition, aging as well as some metabolic disorders accompany a decline in autophagy activity (Aman et al., 2021). Therefore, an intervention to promote autophagy functionality would be helpful to delay or improve the deterioration of body functions and the degenerative conditions of the aging body and may contribute to preventing a variety of disorders. It should be noted that part of the effects of calorie restriction, a well-proven regimen for healthy longevity in animals and humans, is believed to be mediated by the improve-

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ment of autophagy (Morselli et al., 2010). Furthermore, in pharmacological interventions that prolong the health span of animals, such as treatment with rapamycin, an increase in autophagy is a key mechanism involved (Juricic et al., 2022).

Various factors are linked to aging- or to a disease-associated decline in autophagic removal of cellular materials and organelles. Down-regulation of key autophagy components such as beclin-1 and LC3 (Aman et al., 2021) or key autophagy activators such as SIRT1 and AMP kinase (AMPK) (Lee et al., 2008; Salminen and Kaarniranta, 2012) appears to be directly or indirectly related to a decline in autophagy during aging. Accumulation of oxidative damage or elevation of microinflammation, mechanisms that are both strongly implicated in aging and associated diseases as etiological factors, is also associated with autophagy inactivation (He et al., 2018). The flow of autophagy once activated is also prone to impairment, leading to autophagy failure (Zhang et al., 2013). This autophagy flux impairment (AFI) occurs at several different steps during the autophagy process but has been most frequently reported at the step of autolysis, where autophagosomal cargos are degraded by lysosomal hydrolases within autolysosomes, a structure formed by autophagosome-lysosome fusion. AFI, which usually results in a massive accumulation of autolysosomal vacuoles, has been implicated in various neurological disorders (Ntsapi et al., 2016). Furthermore, cell survival is critically affected by autophagy flux, and its impairment plays an important etiological role in certain pathological conditions (Zhang et al., 2013). Therefore, an in-depth understanding of the mechanisms underlying the regulation of autophagy flux and developing a means to intervene in AFI mechanisms are warranted. Although studies on autophagy have mainly focused on understanding the mechanisms underlying its activation, recently, the molecular details of AFI have slowly been unveiled mainly through work on certain cellular stress conditions such as glucose deprivation.

Glucose deprivation is a condition in which cells are not supplied with glucose but are well fed with other nutrients under in vitro or in vivo conditions. In cells starved of glucose, autophagy is activated, but its flux is blocked at the autolysis step, likely due to poor lysosomal acidity (Song and Hwang, 2020). A direct role of glucose in the acidification of the lysosomal lumen has been demonstrated in early studies, mainly in yeast models (Moruno et al., 2012), and therefore the absence of glucose itself may be related to an impairment of lysosomal acidity. However, it was recently found that a high level of reactive oxygen species (ROS) is involved in poor lysosome acidification and autolysosome accumulation (Kang et al., 2017). Cells starved for glucose also exhibit an increase in ROS levels, which is attributed to increased oxidative phosphorylation in mitochondria (Song and Hwang, 2020). Mitochondria are victims and culprits in the imposing of oxidative stress because they both produce ROS and are also damaged by them. Even higher levels of ROS are released from damaged mitochondria, causing even a higher level of damage. Cells subject to ROS-related stress conditions such as replicative senescence and glucose deprivation are easily expected to exhibit this vicious cycle (Kang and Hwang, 2009; Song and Hwang, 2020; Zorov et al., 2014). In fact, upon glucose deprivation, mitochondria undergo a severe change in their mass, structure, and function, suggesting an involvement of mitochondrial change in lysosomal acidification as well as AFI. Therefore, understanding in detail the association of mitochondrial changes to AFI and developing methods to modulate changes in mitochondria could provide an important strategy for intervening in AFI and its associated disease conditions. Herein, information from the literature is summarized to promote a better understanding of the molecular and cellular pathways of AFI based primarily on recent studies investigating lysosomal acidification during glucose deprivation and replicative senescence. Furthermore, the effectiveness of approach to modulate cellular oxidative stress or mitochondrial function on AFI arising from glucose deprivation is discussed.

ACTIVATION OF AUTOPHAGY WITH IMPAIRED FLUX IN CELLS UNDERGOING GLUCOSE DEPRIVATION

Autophagy activation has been well demonstrated in cells in conditions of hypoglycemia in vivo and cells undergoing glucose deprivation in vitro (Karabiyik et al., 2021; Moruno et al., 2012; Ramirez-Peinado et al., 2013; Song and Hwang, 2020). An increase in the activities of either or both SIRT1 and AMPK, which function in autophagy by triggering or facilitating autophagosome formation and autophagosome-lysosome fusion (Hariharan et al., 2010; Huang et al., 2015; Karabiyik et al., 2021; Wang et al., 2022), or inducing inhibition of the negative regulator (Leprivier and Rotblat, 2020), has been observed in cells undergoing glucose deprivation. However, autophagy appears to be successfully executed upon glucose deprivation only briefly. As starvation is prolonged over hours, LC3-positive granules, i.e., autophagosomes and autolysosomes, accumulate. Evidence has accumulated indicating the involvement of poor autophagic degradation rather than a pure increase in autophagy under glucose deprivation. Autolysosomes, which are experimentally identified in confocal microscopy as granules in which fluorescence signals from LC3 and lysosomes are co-localized, increase in number, as shown in Fig. 1 (Glu(-)). The number of lysosomes also increases dramatically due to increased activity of AMPK (Li et al., 2017), and the majority are present as autolysosomes. Furthermore, most lipofuscin granules, which are aggregates of oxidatively modified lipids and proteins trapped in lysosomes, co-localize with autolysosomes, and their number also increases during glucose deprivation, indicating that these autolysosomes are indeed the organelles experiencing defects in cargo digestion (Song et al., 2023). This glucose-starvation induced AFI has been observed to occur in multiple different cell types including human and mouse fibroblasts and cancer-derived cells (Ramirez-Peinado et al., 2013; Song and Hwang, 2020).

Each of the steps in autophagy, cargo recognition, autophagosome enwrapping, autophagosome-lysosome fusion, and autolysosomal degradation has been shown to be subject to interference by the presence of defects or abnormal modifications of proteins involved in that process in diseases and cellular disease models (Ikenaka et al., 2013; Oh et al., 2022; Wong and Holzbaur, 2014). In glucose deprivation, low acidity within the lysosomal lumen is suggested as the

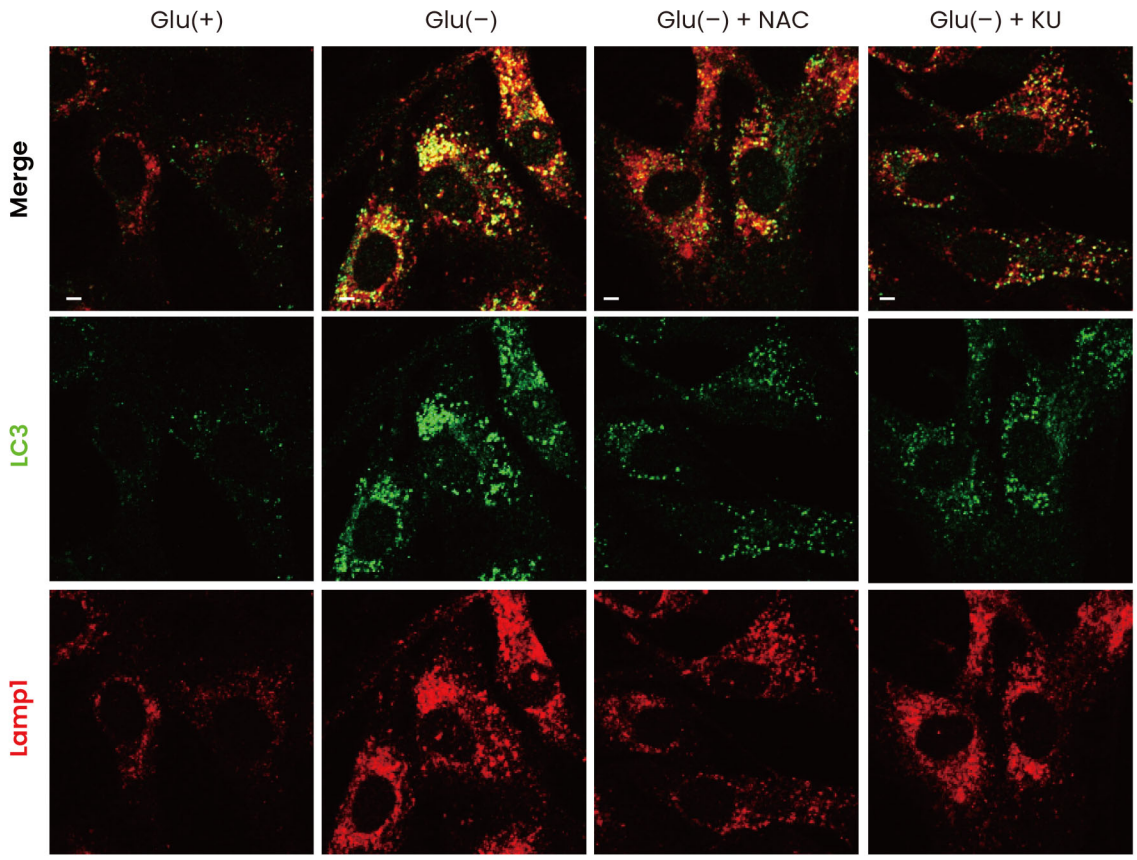

Fig. 1. Accumulation of autolysosome upon glucose deprivation and its attenuation by the supplementation of an anti-oxidant, n-acetyl cysteine (NAC), or an ATM inhibitor, KU60019 (KU). Human fibroblasts were grown in a glucose-free medium for 72 h and then subjected to confocal microscopy after staining with antibodies specific to LC3 and Lamp1 to visualize autophagosomes (green fluorescence) and lysosomes (red fluorescence), respectively. Both autophagosomes and lysosomes increase dramatically in number in glucose-deprived cells (Glu(-)), and most of them co-localize indicating their being autolysosomes. The increase of yellow puncta representing autolysosomes is attenuated by the treatment of NAC or KU60019 during the period of glucose deprivation. Scale bars = 10 μm. Reproduced from Fig. 4C in the article of Song and Hwang (2020) (Biomolecules *10*, 761) under the terms of the Creative Commons Attribution (CC BY 4.0) license.

cause of the poor autolysosomal degradation. Low acidity in the lumen of lysosomes in glucose-deprived cells have been confirmed by measuring the relative acidity of lysosomes using commercially available dyes that sense lysosomal pH (Lysosensor) and also through visual indication of high pH of autolysosomes after transfection of plasmid (ptfLC3) encoding a hybrid LC3 protein that emits different fluorescence depending on pH (Kimura et al., 2007; Song and Hwang, 2020).

MECHANISMS UNDERLYING THE IMPAIRMENT OF LYSOSOME ACIDIFICATION

At least two different cellular routes have been proposed to reduce lysosomal acidity. Mutations in ATP13A2 (PARK9), which encodes lysosomal P5-type ATPase, a putative polyamine transporter, have been reported to be associated with impaired lysosomal acidification and decreased autophagosomal clearance, which is believed to induce accumulation of Lewy bodies associated with neuronal degeneration or dementia (Dehay et al., 2012). Although the possible involvement of poor P5-type ATPase has not been ruled out, the loss or down-regulation of vacuolar H^+-ATPase (V-ATPase), which pumps protons into the lumen of the lysosome, has been better suggested as a reason for low lysosomal acidity in studies on glucose deprivation and is also assumed to be responsible for impaired lysosomal acidity in certain pathological conditions (Kim et al., 2021). As to how glucose deprivation drives a decrease in V-ATPase activity, two mechanisms have been proposed through quite independent studies.

Control by glucose molecules through direct modulation of V-ATPase assembly

V-ATPase, the proton pump which creates the luminal acidity

of lysosomes and endosomes, comprises two multiple subunit domains, V_1, an ATPase, and V_0, a channel responsible for proton translocation (Kane, 1995). In mammalian cells as well as in yeast, the activity of V-ATPase is regulated by the availability of glucose and by cellular energy levels. When glucose is transported into cells, Ras protein signaling is activated (Manchester et al., 1994) and stimulates adenylate cyclase to produce cyclic AMP, which, in turn, triggers activation of protein kinase A (PKA). The assembly of the V-ATPase domain has been proposed to be driven by PKA-mediated phosphorylation of subunit C, a key determinant of the stability of V-ATPase (Alzamora et al., 2010). In the absence of glucose, the V_1 domain dissociates from the V_0 domain, leaving it unable to translocate protons into the lumen of the lysosome (Parra and Kane, 1998). Glucose is also required for the protein, RAVE (regulator of H^+-ATPase of vacuoles and endosomes), which is released from the vacuolar membranes in the absence of glucose, to return to the vacuolar membranes and facilitates the re-integration of subunit C into the V_1 subunit (Smardon and Kane, 2007). The finding that glucose availability determines V-ATPase activity led to the hypothesis that V-ATPase disassembly during glucose deprivation is a cellular function that limits ATP consumption during times of energy stress. Indeed, the disassembly and reassembly of V-ATPase are dependent on the cellular glucose concentration and the flux of glycolysis. When glucose is available, the glycolytic enzymes aldolase and phosphofructokinase interact with V-ATPase, promoting assembly (Chan and Parra, 2014; Kane, 1995; Lu et al., 2007). Interestingly, it has also been reported that in acute glucose deprivation, V-ATPase rapidly assembles and drives acidification of the lysosome (McGuire and Forgac, 2018). In the early phase or during a short period of glucose deprivation (such as 10 min, as determined by McGuire and Forgac, 2018), V-ATPase may be activated for rapid degradation and recycling of resources for ATP generation. However, V-ATPase eventually disassembles, and the lysosomal pH gradually increases during chronic glucose deprivation, such as over a period of several hours. Thus, ATP consumption by V-ATPase pumping protons is suppressed in long-term starvation. This suggests that there might be a switch other than levels of glucose, by which an active form or assembled V-ATPase is inactivated or disassembled. A reduction in cellular ATP levels is not likely a factor since in cells undergoing glucose deprivation, ATP levels do not decrease (Song and Hwang, 2020) as shown in Fig. 2. A factor that may develop under starvation conditions may possibly function as a switch.

High-level ROS-induced V-ATPase inactivation

A second mechanism involves impaired lysosomal acidity due to the high levels of ROS and suggests the possibility that ROS

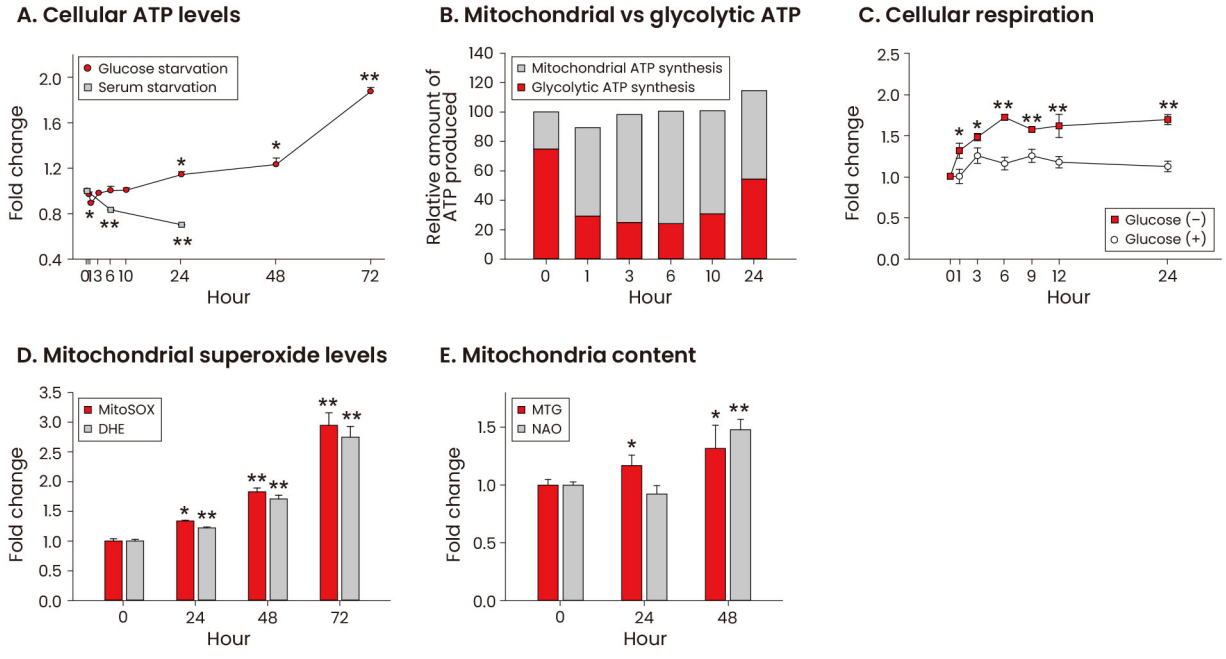

Fig. 2. An increase in cellular ATP levels is driven by increased mitochondrial ATP production and is accompanied by an increase in mitochondrial superoxide generation and mitochondrial content in glucose-deprived cells. (A) During glucose deprivation, there is a gradual increase in cellular ATP content over a 72-h period. (B) After at least 1 h of glucose deprivation, ATP production from mitochondria increases, while far less ATP is produced from glycolysis. (C) Total cellular oxygen consumption increases immediately after glucose deprivation and is maintained for at least 24 h. (D) The levels of mitochondrial (MitoSox) and cytosolic superoxide (DHE) increase substantially in glucose-deprived cells. (E) The increase in mitochondrial content during glucose deprivation as determined by using two different mitochondria-specific fluorescence dyes, MTG and nonyl acridine orange (NAO). Selectively reproduced from Figs. 1, 2, and 4 in the article of Song and Hwang (2018) (Cells *8*, 11) under the terms of the Creative Commons Attribution (CC BY 4.0) license.

levels are a factor that may function as a switch in the V-ATPase inactivation. The involvement of high ROS levels in poor lysosome acidity was first proposed by a study on replicative senescence. Cells undergoing senescence exhibit a high level of β-galactosidase activity, a phenotype called senescence-associated β–galactosidase (SA β-Gal) activity. Previously, this activity was shown to be expressed as a result of an increased expression of lysosomal β-galactosidase (Lee et al., 2006). Increased lysosomal biogenesis has recently been confirmed by increased transcription of lysosomal genes through the increase in nuclear-localized transcription factors TFEB/TFE2 (Curnock et al., 2023). However, this mechanism alone is not fully responsible for the elevated lysosomal content in senescence. Possible impairment of autophagic flux during cellular senescence has been suggested (Jung et al., 2007) and provides an answer. In addition, the involvement of defective V-ATPase was also proposed to explain AFI (Kang et al., 2017). In the latter study, ROS levels increase during the progression of replicative senescence leading to an increase in DNA damage and activation of the ataxia-telangiactasia mutated (ATM) protein, a serine/threonine kinase (Khanna et al., 2001). ATM, a protein majorly functioning in nucleus, has been shown to be activated in the cytoplasm in response to ROS and to phosphorylate certain factors independently of DNA damage (Alexander and Walker, 2010). The ATP6V1G1 subunit of V-ATPase appears to be one (Kang et al., 2017). Importantly, phosphorylation of ATP6V1G1 interferes with its interaction with ATP6V1E1, which is necessary for the assembly of subunit V1 (Fethiere et al., 2004), leading to its disassembly (Kang et al., 2017). As an outcome, V-ATPase is inactivated and acidification of lysosomes is impaired. Exposure to KU60019, an inhibitor of ATM, in senescent human fibroblasts resulted in an increase in lysosome acidity along with a recovery of lysosome function (Kang et al., 2017). Senescence is attenuated as determined by increased cell division and decreased SA β-Gal activity, surprisingly suggesting that cellular senescence may be a reversible phenomenon. Furthermore, this change was also accompanied by the restoration of autophagy flux. These findings provided a better understanding of the nature of SA β-Gal activity and also the molecular cause for the increase in lysosomal mass in senescent cells: the result of an AFI caused by the failure of lysosomal acidification driven by high ROS levels. In addition, the increase in lysosome biogenesis might be interpreted as an effort by the cell to quantitatively compensate for the dysfunction.

Cellular mechanisms of glucose deprivation are quite analogous in many aspects to those of cellular senescence. Importantly, in glucose-deprived cells, ROS levels produced by mitochondria appear to increase substantially as shown in Fig. 2D, to induce ATM activation (Ahmad et al., 2005; Song and Hwang, 2018). ATM activated in the cytoplasm rapidly engages in LKB1-AMPK activation to suppress mTORC1 signaling, and thus activate autophagy (Alexander and Walker, 2010). Importantly, treatment with n-acetyl cysteine (NAC), a potent antioxidant chemical, attenuated the impairment of lysosomal acidity and autophagic flux as shown in Fig. 1 (column 'Glu(-)+NAC') (Song and Hwang, 2020). Treatment of KU60019 also reduced the levels of autolysosomes (Fig. 1, column 'Glu(-)+KU'). These suggest a key role for ROS and thereby the activation of ATM in lysosomal acidity impairment and in AFI in glucose-deprived cells. In addition to cytoplasmic ATM activation, ROS might impose further hindrance to lysosomal acidity by activating the extracellular signal-regulated kinase (ERK) pathway (Son et al., 2011), which is also involved in the activation of autophagy (Son et al., 2013). Indeed, ERK is activated in glucose-deprived cells (Lee et al., 1998; Song and Hwang, 2020). Treatment with an ERK inhibitor can partially restore lysosomal acidity and autophagic flux (Song and Hwang, 2020). Altogether, these findings strongly argue for the possibility that lysosome acidity impairment through V-ATPase inhibition might be a passive event simply driven by high-level ROS rather than an outcome of the cellular effort to cope with scarcity of the glycolytic substrate.

Meanwhile, in a study of cellular senescence, KU60019 treatment switched mitochondrial metabolic reprogramming toward oxidative phosphorylation (Kang et al., 2017). The senescent fibroblasts observed in the study presented higher oxygen consumption as well as a greater dependence on glycolysis to meet energy demands, but following KU-60019 treatment, these requirements were reduced to those of early-passage fibroblasts; the proportion of ATP generation from glycolysis was decreased while that from oxidative phosphorylation increased. This change in mitochondrial function was assumed to be a prerequisite for KU60019 to attenuate senescence, although the study did not examine how this metabolic shift was caused. Whether the change in AFI status induced by KU60019 treatment caused a change in mitochondrial status or vice versa is of importance. Compared to the rather rapid change in V-ATPase activity and lysosomal acidity expected after ATM inhibition, these mitochondrial-associated changes occurred rather slowly (Kang et al., 2017). This suggests that mitochondrial changes may occur as an outcome of the alleviation of autolysis impairment.

IMPAIRED MITOPHAGY AGGRAVATES OR CAUSES LYSOSOMAL ACIDITY FAILURE

Differently from an initial belief described in early studies (Buzzai et al., 2005; Hardie, 2003; Liu et al., 2003), cellular ATP level does not decline in cells undergoing glucose deprivation. Although glycolytic ATP production decreases, mitochondrial ATP generation increases dramatically (Ahmad et al., 2005; Jelluma et al., 2006; Song and Hwang, 2018) as shown in Fig. 2B.

Functional and structural changes of mitochondria in glucose-deprived cells

In fact, as glucose deprivation continues, mitochondria undergo a dramatic change in function and structure, and fatty acid β-oxidation increases. And, oxidative phosphorylation also increases as indicated by the elevation of cellular respiration (Ahmad et al., 2005; Buzzai et al., 2005; Jelluma et al., 2006; Swerdlow, 2009) and as shown in Fig. 2C, supporting enhanced mitochondrial ATP generation in compensation for reduced glycolytic ATP generation. The mitochondrial content also increases in parallel (shown in Fig. 2E), providing a basis

for the higher levels of ATP and superoxide generation (shown in Fig. 2). This increase in mitochondrial content has been reported to be caused by SIRT1 activity-induced mitochondrial biogenesis (Song and Hwang, 2018). In glucose-deprived cells, SIRT1 activity is enhanced through an increase in the NAD^+/NADH ratio driven by a decrease in glycolytic reduction of NAD^+ to NADH and believed to drive mitochondria biogenesis along with various other changes (Brenmoehl and Hoeflich, 2013). Importantly, in healthy cells, mitochondrial biogenesis is balanced by their degradation, which together determines mitochondrial quality (Ploumi et al., 2017). However, this balance is not maintained in glucose-deprived cells. Mitochondria appear to resist degradation. Along with this increase in cellular mitochondrial content, mitochondria have also been shown to get elongated (Song and Hwang, 2018). This implies failure of mitophagy, either cause or effect, the important consequence of which would be enhancement of ROS production (Twig et al., 2008). In cells undergoing replicative senescence, the mitochondria are elongated and also resist mitophagy (Kang and Hwang, 2009; Miwa et al., 2022).

Mitophagy failure driven by increased mitochondrial ATP generation

An increase in mitochondrial ATP production drives AFI through a concomitant increase in ROS levels. It appears that a factor is actively involved in this and plays a role in exacer-

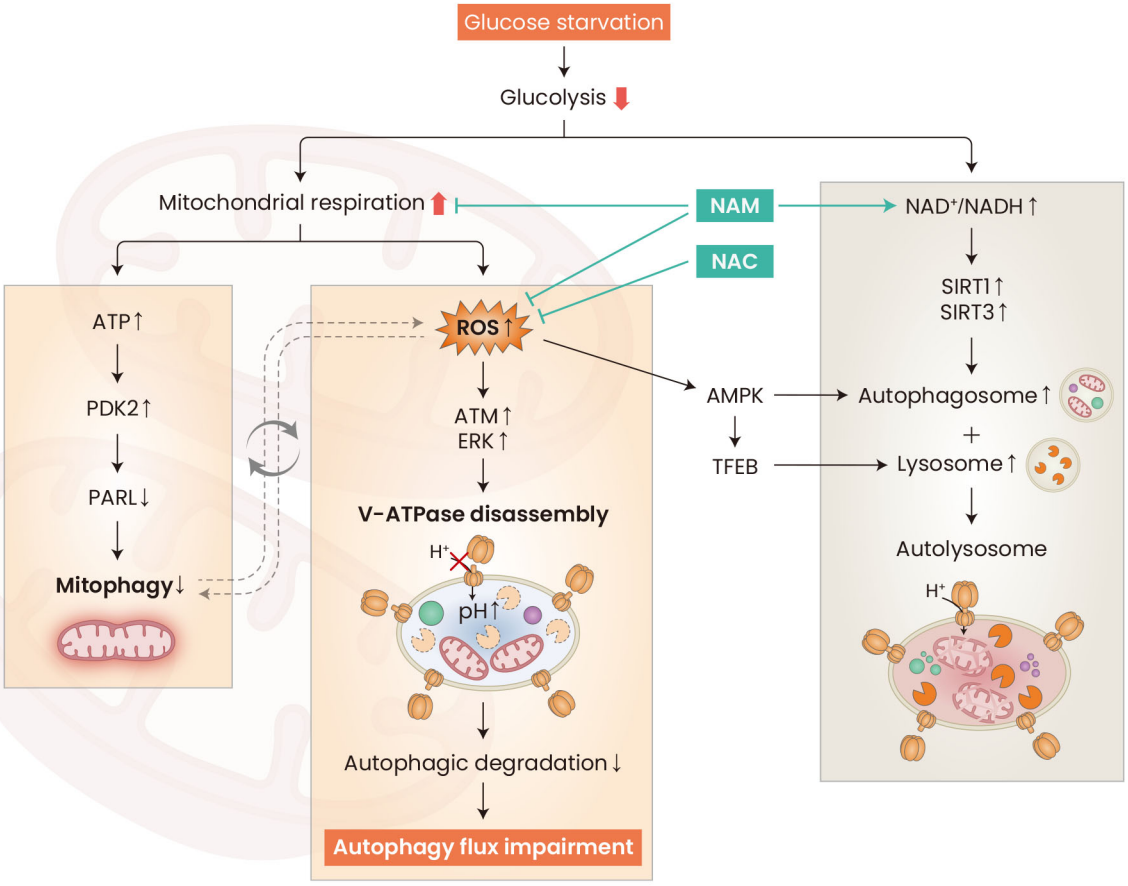

Fig. 3. Pathways to autophagy flux impairment (AFI) in glucose-deprived cells and putative effects of nicotinamide (NAM) and n-acetyl cysteine (NAC). Upon glucose deprivation, the scarcity of glycolytic pyruvate feed results in an increase in mitochondrial respiration and ATP production. Activity of PDK2 also increases and causes a decrease in β-cleavage of PARN and PINK/Parkin-mediated mitophagy. This results an increased mitochondrial mass and generation of reactive oxygen species (ROS), through which a vicious cycle of mitochondrial ROS generation is eventually formed (indicated by the red circle with arrows). Increased ROS levels activate ATM and ERK, which cause the disassembly of V-ATPase and loss of lysosomal acidity. Therefore, lysosomes that increase upon ROS activation of AMPK exhibit low acidity. Together, this leads to poor degradation in autolysosomes and impaired autophagy flux. NAM treatment leads to elevated mitochondrial levels of NAD^+/NADH, which reduces mitochondrial respiration and ATP generation. This increase in NAD^+/NADH levels further activates SIRT1 and SIRT3, which collaborate to induce mitophagy and enhance mitochondrial quality. Consequently, NAM supplementation is expected to alleviate AFI by mitigating ROS generation. Meanwhile, treatment of NAC has demonstrated the ability to attenuate AFI in glucose-deprived cells, highlighting the effectiveness of reducing ROS stress in attenuating AFI. The expected pathways of the effectiveness of NAM and NAC are indicated in blue letters and lines.

bating the problem. Pyruvate dehydrogenase kinase (PDK) 2 phosphorylates and inhibits pyruvate dehydrogenase in response to elevated mitochondrial level of ATP, and thereby downregulates acetyl-CoA feed to TCA cycle while facilitating fatty acid oxidation (Foster, 2012). Under conditions of glucose deprivation, the activity of PDK2 increases due to the scarcity of pyruvate, a negative regulator. PDK2 also phosphorylates an inner mitochondrial membrane protein, presenilin-associated rhomboid-like protein (PARL), which requires β-cleavage, more specifically an autocleavage at its N-terminus, to function in mitophagy by facilitating efficient PINK-mediated PARKIN recruitment to damaged mitochondria (Shi et al., 2011). PDK2-mediated phosphorylation attenuates the β-cleavage of PARL, and thereby, down-regulates mitophagy (Shi and McQuibban, 2017). Therefore, increased mitochondrial ATP levels result in down-regulation of PINK/PARKIN-mediated mitophagy, a mechanism that specializes in removing damaged parts of mitochondria (Ashrafi and Schwarz, 2013). Together, in low levels of glucose availability, PDK2 functions to drive a high levels generation of mitochondrial ATP via activation of fatty acid oxidation and oxidative phosphorylation and downregulation of mitophagy. The accompanying elevation of ROS generation from increased mass of mitochondria would aggravate V-ATPase inactivation leading to lysosomal acidity impairment and AFI. The putative pathway that involves PDK2 function in mitophagy is summarized in Fig. 3. Importantly, it is suggested that reducing oxidative phosphorylation and mitochondrial mass, or suppressing the generation of ATP and/or ROS, may be successful in attenuating AFI.

CONCLUSION

The studies performed to date strongly suggest that high levels of ROS induce AFI by suppressing V-ATPase in lysosome membrane through the activation of pathways involving ATM and ERK. It is also suggested that, under conditions of poor glycolysis, cells, aided by PDK2, also are forced to rely on oxidative phosphorylation and produce high levels of ATP along with ROS. And, PDK2-mediated downregulation of mitophagy and thereby of mitochondria quality drives higher ROS levels, which then further aggravates AFI. Therefore, in cells undergoing a combination of high levels of ROS and low levels of mitophagy, lysosomal acidity impairment is expected to be significantly exacerbated. This suggests that AFI in glucose-deprived cells can be alleviated by reducing ROS levels. Indeed, the treatment of NAC was shown to significantly attenuate the increase of autolysosomes (Figs. 1 and 3). Therefore, anti-oxidative therapies might be helpful in preventing or alleviating disease associated with AFI. Meanwhile, since ROS are mainly produced from dysfunctional mitochondria, cells suffering impaired mitophagy or poor mitochondrial quality as well are expected to experience AFI; however, AFI might have been unnoticed in such cells since autophagy was not activated highly enough to be noticed. Therefore, mitochondria structural dynamics and active mitophagy might be importantly working in suppressing AFI in normal cellular life (Nakamura and Yoshimori, 2018; Yoo and Jung, 2018). Furthermore, means to activate mitophagy can be helpful in preventing AFI-associated diseases. In this context, it is noteworthy to mention a possible effectiveness of the treatment of nicotinamide (NAM), a precursor of nicotinamide adenine dinucleotide (NAD^+). NAM has been shown to exert various beneficial effects on cells including expansion of replication and differentiation capacities. An important mechanism of NAM's effectiveness involves a marked suppression of mitochondrial ROS generation, which is attributed to enhanced mitochondrial structural dynamics mediated by SIRT1 activation as indicated in Fig. 3 (Jang et al., 2012; Kang and Hwang, 2009; Kwak et al., 2015). Therefore, the application of NAM (as well as possibly other NAD^+ precursors) and other chemicals with pro-mitophagic potential can be considered as therapeutics in preventing or slowing the progression of AFI-related diseases. Overall, this study presents an important clue for managing AFI and mitigating associated issues in relevant diseases.

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AUTHOR CONTRIBUTIONS

S.B.S. and E.S.H. conceived and designed the study. S.B.S. performed experiments evaluating the effects of nicotinamide. E.S.H. took the leading role in writing the manuscript and creating figures. Both authors reviewed, revised, and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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